REMARKS

Claims 1-21 are pending. Claims 1-21 have been rejected. Claim 1 has been amended to correct the term "sample" to "analyte". Support for the amendment can be found throughout the Claims and the specification, for example, Claim 1 and paragraphs **0033** and **0042**. Claim 1 was further amended to more explicitly state that the method of illumination of the fluorophore is via direct illumination. Support for the amendment can be found throughout the Claims and the specification, for example, paragraphs **0045**, **0070**, **0005**, **0006**, **0090**, **0060**, and **0092**. Applicants would also note that the disclosed embodiments discussing beads involve direct illumination. The amendments add no new matter.

Rejections under 35 U.S.C. §112, Definiteness

Claim 1 stands rejected as indefinite. The Examiner asserts that it is not clear if step (d) "is intended to mean that if the fluorescence anisotropy measurement in the presence of a sample is greater than when there isn't a sample, then presence or amount is identified, or if the step is intended to mean that... a greater anisotropy measurement indicates the presence or an amount of an analyte." Applicants thank the Examiner for pointing out the typographical error and have amended Claim 1 accordingly.

Claims 4 and 5 stand rejected because the Examiner asserts that the term "about" is a relative term which renders the claim indefinite. Applicants respectfully direct the Examiner's attention to § 2173.05(b)(A) of the M.P.E.P., which states:

> The fact that claim language, including terms of degree, may not be precise, does not automatically render the claim indefinite under 35 U.S.C. 112, second paragraph. *Seattle Box Co., v. Industrial Crating & Packing, Inc.*, 731 F.2d 818, 221 USPQ 568 (Fed. Cir. 1984).... The term "about" used to define the area of the lower end of a mold as between 25 to about 45% of the mold entrance was held to be clear.... *Ex parte Eastwood*, 163 USPQ 316 (Bd. App. 1968). Similarly, in *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), the court held that a limitation defining the stretch rate of a plastic as "exceeding about 10% per second" is definite because infringement could clearly be assessed through the use of a stopwatch.

Furthermore, Applicants remind the Examiner that the "acceptability of the claim language depends on whether one of ordinary skill in the art would understand what is claimed, in light of the specification." (M.P.E.P. § 2173.05(b)(A). In the present situation, one of ordinary skill in

the art would understand the term "about" in claims 4 and 5 to refer to the inherent uncertainty and variability in any measurement of size. (*See e.g.,* BJ Services Co. v. Halliburton Energy Serv. Inc., 67 U.S.P.Q.2d 1692, 338 F.3d 1368 (Fed. Cir. 2003) ("About 0.06" viscosity is definite under §112, 2nd paragraph, because, to the skilled artisan, "about" is intended to encompass the range of experimental error that occurs in any measurement). Similarly, in the present case, the term "about" is meant to encompass the standard variability in measuring the spheres. Applicants would point out that the use of "about" in this situation adds certainty and clarity to the claims because an exact measurement of any distance is impossible to achieve in actual practice.

Additionally, Applicants direct the Examiner's attention to paragraph **0057** of the present application, in which the size of the microspheres is also discussed.

Rejections under 35 U.S.C. §102

Several of the Claims stand rejected under 35 U.S.C. §102 as anticipated. Section 2143 of the M.P.E.P. states that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California,* 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Furthermore, "[t]he identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

As a general point, Applicants direct the Examiner's attention to the fact that Claim 1 recites that the anisotropy measurement is greater in the presence of the analyte than the anisotropy measurement in the absence of the analyte. This limitation, an increase in anisotropy upon binding, has not been identified in any of the references cited as the basis for the rejections as set forth in the current Office Action. As all elements of the claims must be taught in order to support rejections under §102 and §103, and as the cited references fail to teach all elements of the claims, Applicants request that the rejections be withdrawn.

Rejection under 35 U.S.C. §102(b) Potyrailo et al.

Claims 1, 8, and 16 stand rejected under 35 U.S.C. §102(b) as being anticipated by Potyrailo et al. (Potyrailo et al., Anal Chem, 70:3419-3425 (1998), herein after "Potyrailo"). It is

asserted that Potyrailo teaches an anti-thrombin DNA aptamer immobilized to a glass surface and the use of fluorescence anisotropy to detect analyte binding through the use of a vertically polarized laser. Applicants respectfully traverse.

Present Claim 1, from which all other claims depend, recites illuminating the aptamer with polarized light. As the fluorophore is attached to the aptamer, the fluorophore is also illuminated with polarized light. Thus, the fluorophore is directly illuminated by polarized light while the fluorophore is immobilized on a support. In order to further emphasize this point, Claim 1 has been amended to include the term "directly" as regards to how the illuminating light contacts the aptamer and fluorophore. Thus, the aptamer and fluorophore of Claim 1 are not illuminated via an evanescent wave, TIR, surface plasmon resonance, or some other indirect method. Moreover, concerning other claimed embodiments, which involve the attachment of the fluorophores to beads, Applicants are not aware of how an evanescent wave, or other indirect method, could be used in such curved support situations.

Applicants respectfully point out that this method of direct illumination of a fluorophore attached to a support is in sharp contrast to what is pointed out in Potyrailo, which requires the use of an evanescent-field to illuminate the fluorophore instead of directly illuminating the fluorophore when the fluorophore is attached to a support (see abstract, Figure 1, and the paper generally). Potyrailo does not anticipate independent Claim 1 because Potyrailo does not teach directly illuminating a fluorophore that is attached to a support. As Claims 8 and 16 depend from Claim 1, Claims 8, and 16 must also be novel.

Rejection under 35 U.S.C. §102(e) Gold et al.

Claims 1, 9, 11, 12, 14, and 17-21 stand rejected under 35 U.S.C. §102(e) as being anticipated by Gold et al. (U.S. Pat. No.: 6,544,776, hereinafter "Gold") in light of Fang et al. (Anal. Chem., 73:5752-5757, (2001), hereinafter "Fang"). It is asserted that Gold teaches aptamers immobilized to the surface of biochips and measurement of fluorescence anisotropy to determine the presence of target molecules. It is also asserted that Fang defines anisotropy as requiring the use of polarized light. It is thus asserted that one of skill in the art would logically combine these two references, allegedly because polarized light is required for measuring anisotropy. Applicants respectfully traverse.

Applicants note that it is asserted that one of skill in the art would effectively combine or equate the two references because Gold teaches a method involving anisotropy and Fang teaches a method of measuring anisotropy. This implies that there is an assumption that all methods for monitoring or determining anisotropy are interchangeable. This assumption is incorrect.

In particular, Gold, like Potyrailo, is directed to one method of measuring anisotropy (support-based), while Fang is directed to another method of measuring anisotropy (solution-based). Each of these two methods involves different issues. Thus, in each method, anisotropy is measured in a different way. Applicants respectfully remind the Examiner that anisotropy effectively measures aspects of mobility of a fluorophore. As will be appreciated by one of skill in the art, a fluorophore that is freely rotating and diffusing through a solution will have a dramatically different anisotropy measurement than one that is attached to a support and thereby relatively immobilized.

In fact, Fang teaches that the change in anisotropy in his solution-based system is dependent upon the entire molecule rotating freely in solution. For example, "[w]hen the labeled aptamer is bound with its target protein, the rotational motion of the fluorophore attached to the complex becomes much slower because of an increased molecular weight after binding, resulting in a significant fluorescence anisotropy change." (Fang, Abstract). Additionally, Fang states that "[a]ptamers are relatively small molecules, so the binding of target proteins will bring a significant change in their molecular weights and, therefore, their rotational diffusion rates of the labeled fluorophores, resulting in detectable variations in their fluorescence anisotropy measurements." (p. 5753, Col. 2). Thus, it is clear that the method of Fang appears to require aptamers and fluorophores that are capable of free rotational diffusion.

This is in contrast to a situation in which a fluorophore is immobilized to a support. In this later scenario, a different technique (involving an evanescent field) is used in the cited references to detect any changes in anisotropy. To put it another way, the system described in Fang is applicable to situations in which one has a system where binding of the aptamer to a target will result in a large percent change in the mass of the aptamer resulting in a greater change in anisotropy which can be readily detected. Such a system would not be expected to work if the aptamer was very large and the target binding to the aptamer did not result in a large change in mass. This is important because the immobilization of the aptamer to a support can effectively give the aptamer the mass of the support, which is a relatively huge mass. One of

skill in the art would not expect that direct illumination of the aptamer and fluorophore would work with a support bound aptamer and fluorophore.

Applicants submit that one of skill in the art would not use a method for measuring solution-based changes in anisotropy to measure support-based changes in anisotropy, especially when the art does not consider the techniques to be interchangeable and no one has taught that they are interchangeable. Indeed, as discussed in more detail below, Applicants submit that such a combination is inadequate, even under a 35 U.S.C. §103 analysis.

Additionally, Applicants note that Gold explicitly teaches a method of measuring support-based anisotropy, via an evanescent field, similar to that in Potyrailo (Col. 19, lines 15-47). Applicants can imagine no reason why one would ignore the explicit teachings of Gold, which are applicable to support-based anisotropy measurements.

In light of the above arguments, Applicants request that the rejection under 35 U.S.C. §102(e) be withdrawn and Claims 1, 9, 11, 12, 14, and 17-21 be allowed.

Rejection under 35 U.S.C. §102(b) Hesselberth et al.

Claims 1, 8, 9, and 11-15 stand rejected under §102(b) as being anticipated by Hesselberth et al. (Rev. Mol. Biotech., 74, 15-25 (2000), hereinafter "Hesselberth") in light of Fang. It is asserted that Hesselberth teaches "signaling aptamers... geared toward isolating individuals with particular attributes from the random sequence population (p. 16, col. 1) where the aptamers are synthetically labeled with fluorescein (p. 19, col. 2) and then immobilized on a glass surface." (Office Action, page 5). Furthermore, it is asserted that Fang demonstrates that one of skill in the art would have known to use polarized light in this situation to measure a change in anisotropy. Applicants respectfully traverse.

As explained above, at best, one of skill in the art would have used an indirect method (*e.g.*, an evanescent field) to illuminate the fluorophore rather than the direct illumination of the fluorophore, as the system in Hesselberth is immobilized on a flat glass surface or support.

Moreover, Hesselberth explicitly teaches the use of "surface plasmon resonance spectroscopy" (p. 19, col. 2) which is an indirect method of illuminating the fluorophore. As above, Applicants assert that one of skill in the art would not have ignored the teachings of Hesselberth, a support-based method. Reference to Fang is improper, as Fang is a solution-based technique. One of skill in the art would, at best, use the method taught in Hesselberth itself,

which is an indirect method of illumination. The present claims are directed to anisotropy measurements using direct illumination. Therefore, the above "combination" cannot anticipate the present claims.

Applicants respectfully request that the rejection be withdrawn and claims 1, 8, 9, and 11-15 be allowed.

Rejections under 35 U.S.C. §103(a)

The Examiner has rejected several of the claims under 35 U.S.C. §103 as being obvious in view of the combination of various references in light of additional references, each of which is directly addressed below. Initially we note that section 2143 of the M.P.E.P. recites the three basic requirements for establishing a *prima facie* case of obviousness. First, the cited reference (or references when combined) must teach or suggest all the claim limitations. Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Finally, there must be a reasonable expectation of success.

As mentioned above, Applicants note that the previous Office Action did not supply a reference that teaches that an increase in anisotropy indicates the presence of an analyte. Since the cited references fails to teach or suggest all of the claim limitations, Applicants request that the rejections under §103 be withdrawn.

Rejections under 35 U.S.C. §103(a) Lee et al in view of Potyrailo et al.

Claims 1-7, 9, 11-13, 16, and 19 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al. (Anal. Biochem, 282:142-146, (2002), hereinafter "Lee") in view of Potyrailo. Potyrailo is directed to an evanescent-wave induced anisotropy measurement (See, Col. 1, p 3421). It is asserted that Lee's teaching of microbeads coated with aptamers would be sufficient, when combined with the teaching of Potyrailo, to teach the elements of the current claims. Applicants respectfully traverse.

Applicants first note that not every element recited in the claims is taught by the combination of Lee and Potyrailo. In particular, amended Claim 1 requires that the illumination

of the fluorophore be through direct illumination of the fluorophore, rather than illumination through the use of an evanescent-wave, which is what is taught in Potyrailo (see Figure 1) when the fluorophore is attached to a support. Lee also does not teach the direct illumination of the fluorophore to measure anisotropy, as Lee does not teach measuring anisotropy.

Applicants also note that the motivation is inadequate for combining the two references. The two "advantages" or motivations that the Examiner has used to support this combination apply to evanescent-wave excitation, not direct illumination. However, as described below, evanescent wave illumination is not going to occur in a bead.

Additionally, Applicants respectfully point out to the Examiner that any such motivation concerning the forms of illumination would be inadequate because "[i]f [a] proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. (*In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984)" (M.P.E.P. §2143.01). In this case, since the anisotropy measurements in Potyrailo's support bound aptamers rely upon the generation of an evanescent wave off of a flat support and since the beads in Lee have no flat support, it appears that the combination would prevent Potyrailo's system from generating an effective evanescent wave. Because this combination would prevent Potyrailo's system from functioning as designed, there is no motivation to make the proposed combination.

Finally, Applicants note that there would be no expectation of success for the proposed combination. There is no teaching of how an evanescent wave, as taught in Potyrailo, could be generated in a microbead. Without such a teaching, and without an alternative means of illuminating a fluorophore and detecting a change in anisotropy of a fluorophore that is attached to a support, one of ordinary skill in the art would not have expected that a change in anisotropy would be detected from the binding of a target to an aptamer. Applicants submit that there can be no expectation of success of the combination suggested by the Examiner.

Thus, a *prima facie* case of obviousness has not been established. As such, Applicants request that the rejection be withdrawn and Claims 1-7, 9, 11-13, 16, and 19 allowed.

Rejections under 35 U.S.C. §103(a) Lee et al in view of Fang et al.

Claims 1-9, 11-13, 17-19, and 21 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Lee in light of Fang. It is asserted that Lee teaches a method of measuring an

analyte using DNA aptamers immobilized on the surface of silica beads and making fluorescent measurements. It is admitted that Lee does not teach illumination with polarized light or anisotropy measurements. It is asserted that Fang teaches the detection of PDGF via fluorescence anisotropy using polarized light and that the use of fluorescence anisotropy is expected to be sensitive, convenient and selective. Applicants respectfully traverse.

As discussed in detail above, Fang is directed to a solution-based system and Lee is directed to a support-based system. Because of this difference, the reasons discussed above for lack of motivation to combine and an absence of an expectation of success also apply here.

Additionally, there would be no expectation of success for the combination of 1) a solution-based anisotropy detection system, which relies on diffusion and freedom of rotation of the aptamer (Fang), and 2) a support-based fluorescence detection system in which anisotropy is not measured (Lee). Nothing in Lee suggests how one can attach a fluorophore to a support with an aptamer so that the binding of a target to the aptamer will result in a detectable change in anisotropy of the fluorophore. As discussed above, the change in anisotropy discussed in Fang involves a change in the amount of rotation and diffusion of the aptamer. This is a function of the mass of the aptamer and the change in anisotropy is related to the percent change in mass of the aptamer. Thus, a large percent change in the mass of the aptamer will result in a large change in anisotropy, which will allow for the detection of the binding of the target to the aptamer. On the other hand, if the aptamer is very large and the target is very small, the binding of the aptamer to the target will not result in a large change in mass, which, theoretically, will not result in much of a change in anisotropy. As discussed above, the linking of the aptamer to a glass cover slip can effectively raise the mass of the aptamer to that of the glass cover slip, thus making any change in anisotropy due to target binding unlikely.

As neither of the references supply any reason to believe that an anisotropy change can be observed through direct illumination following the binding of the target to the aptamer when the aptamer is attached to a support, and as the previous discussion and the other references identify this as a problem, Applicants submit that one of skill in the art would not have expected the proposed combination to work.

Thus, a *prima facie* case of obviousness has not been established. As such, Applicants request that the rejection be withdrawn and Claims 1-9, 11-13, 17-19, and 21 allowed.

Rejections under 35 U.S.C. §103(a) Potyrailo et al in view of Fang et al.

Claims 17-19 and 21 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Potyrailo in view of Fang. It is asserted that Potyrailo teaches a method for detecting an analyte in a sample. It is further asserted that Fang teaches the detection of PDGF using fluorescence anisotropy and that the use of fluorescence anisotropy is expected to be sensitive, convenient and selective. Applicants respectfully traverse.

Applicants note that Claims 17-19 and 21 depend from Claim 1 which is novel and nonobvious. Therefore Claims 17-19 and 21 are also novel and nonobvious.

Additionally, Applicants submit that, as described above, one of skill in the art would not have been motivated to combine the solution-based Fang technique with a substrate-based technique and would not have expected the particular combination to work. According to Fang, aptamers are suited for anisotropy measurements because they are "relatively small molecules, so the binding of target proteins will bring a significant change in their molecular weights and, therefore, their rotational diffusion rates of the labeled fluorophores, resulting in detectable variations in their fluorescence anisotropy." (Col. 2, page 5753) From this teaching, it is clear that the minimal size and free movement of the aptamer is what is desirable and what appears to allow the technique in Fang to function. As discussed above, the attachment of the aptamer to a substrate will adversely impact both of these properties. Thus, one of skill in the art would not have wanted to make such a combination and would not have expected the combination to still produce detectable changes in anisotropy upon target binding.

Rejections under 35 U.S.C. §103(a) Lackowicz et al in view of Spridonova et al.

Claims 1-6 and 8-10 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Lakowicz et al. (Anal. Biochem., 267:397-405 (1998), hereinafter "Lakowicz") in view of Spiridonova et al. (Biochem., 67:706-709 (2002) hereinafter "Spiridonova"). It is asserted that Lakowicz teaches measurements of steady-state anisotropies in the presence of reference fluorophores with known anisotropies using a protein binding sensor, providing a weighted average of the anisotropies of the emitting species, and using a laser that was vertically polarized. Lakowicz does not specify that the sensors are fluorophore-labeled aptamers bound to a solid support. It is asserted that Spiridonova teaches the use of porous silica microspheres with immobilized DNA aptamers. Applicants respectfully traverse.

Lakowicz teaches a solution-based detection system (see scheme 1) which uses a solid reference sample as a type of control reference. As such, Lakowicz supplies a similar teaching to that of Fang and a similar inadequacy as well. Spiridonova teaches aptamers on microspheres. As discussed above, Applicants note that the present claims involve the direct illumination of a support-based fluorophore to measure the fluorophore's anisotropy. In contrast, Lakowicz involves the measurement of a solution-based fluorophore. Lakowicz does not address the problems identified above associated with such a combination, such as the reduction in rotational diffusion and any change in anisotropy. As Spiridonova does not address anisotropy issues at any level, Spiridonova does not overcome Lakowicz's failing. As there is no teaching as to why one of skill in the art would have combined a support-based approach and a solution-based approach and as there is no showing that they would have expected the combination to work, Applicants submit that a *prima facie* case of obviousness has not be established.

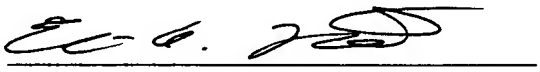
Conclusion

Applicants respectfully submit that for the above-recited reasons the rejections should be withdrawn. Applicants respectfully submit that the present application is in condition for allowance. If, however, some issue remains, the Examiner is cordially invited to telephone the undersigned in order to resolve such issue promptly. Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 11/12/04

By: 

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